INSTRUCTIONS

Pierce® Direct Magnetic IP/Co-IP Kit

88828 2321.1

Number	Description
88828	**Pierce Direct Magnetic IP/Co-IP Kit,** contains sufficient reagents to perform 40 reactions using 25µL of magnetic beads

Kit Contents:

Pierce NHS-Activated Magnetic Beads, 1mL supplied at 10mg/mL in *N*,*N*-dimethylacetamide (DMAC)

IP Lysis/Wash Buffer, 2 × 50mL, pH 7.4, 0.025M Tris, 0.15M NaCl, 0.001M EDTA, 1% NP40, 5% glycerol

Elution Buffer, 5mL, pH 2.0

Neutralization Buffer, 0.5mL, pH 8.5

Quenching Buffer, 25mL, 3M ethanolamine, pH 9.0

Borate Buffer, 1mL, 0.67M

BupH™ Borate Buffer Pack, 0.05M sodium borate, pH 8.5 when dissolved in a final volume of 500mL of ultrapure water

Lane Marker Sample Buffer, Non-reducing, (5X), 5mL, 0.3M Tris•HCl, 5% SDS, 50% glycerol, lane marker tracking dye; pH 6.8

Note: Before using, refer to the product label for the expiration date.

Storage: Upon receipt store at 4°C. Product shipped with an ice pack.

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Introduction

The Thermo Scientific Pierce Direct Magnetic IP/Co-IP Kit enables efficient antigen immunoprecipitations (IP) and co-immunoprecipitations (co-IP). The activated magnetic beads contain *N*-hydroxysuccinimide (NHS) functional groups, which react with primary amines on antibodies to form stable amide linkages. The antibody is first coupled to the NHS-activated magnetic beads in an amine-free buffer. The antibody-coupled beads are washed to remove any non-covalently bound antibody and quenched. The prepared beads are incubated with the antigen sample, washed to remove non-bound material and eluted in a low-pH elution buffer to dissociate the antigen. The kit includes optimized buffers that minimize nonspecific binding and maximize antigen yield. Lane marker sample buffer is included for preparing samples for SDS-PAGE analysis. The beads are manually removed from the solution with a magnetic stand or by automation using an instrument such as the Thermo Scientific KingFisher Flex System. Automated instruments are especially useful for large-scale screenings of multiple samples.

Table 1. Characteristics of Thermo Scientific Pierce NHS-Activated Magnetic Beads.

Composition:	*N*-hydroxysuccinimide (NHS) functional groups on a blocked magnetic bead surface
Magnetization:	Superparamagnetic (no magnetic memory)
Mean Diameter:	1µm (nominal)
Density:	2.0g/cm^3
Bead Concentration:	10mg/mL in DMAC
Binding Capacity:	≥ 26µg of rabbit IgG/mg of beads

Procedure Summary

1. Wash the beads with ice-cold 1mM HCl.
2. Bind antibody to the beads for 30-60 minutes.
3. Wash the beads twice with Elution Buffer.
4. Quench the reaction for 30-60 minutes with Quenching Buffer.
5. Wash the beads once with Modified Borate Buffer and once with IP Lysis/Wash Buffer.
6. Incubate cell lysate with antibody-bound beads for 2 hours at room temperature or overnight at 4ºC.
7. Wash the beads twice with IP Lysis/Wash Buffer and once with ultrapure water.
8. Elute bound antigen.

Important Product Information

- Magnetic beads are moisture-sensitive. To protect the beads, cap the bottle immediately after removing the slurry and wrap lab film around the cap before storing at 4ºC.
- Do not centrifuge, dry or freeze the magnetic beads. Bead aggregation and loss of binding activity may result from using these methods.
- Estimate the amount of protein coupled to the magnetic beads with a protein assay (e.g., Thermo Scientific Pierce 660nm Protein Assay, Product No. 22660 and 22662) and subtract the amount of flow-through protein from the loaded protein. To measure the amount of protein on the bead directly, use the Thermo Scientific Pierce Micro BCA Protein Assay (Product No. 23235, on our website see Tech Tip #75: Measure protein bound to Pierce NHS-Activated Magnetic Beads).
- For coupling antibodies to magnetic beads, ensure the antibody storage solution does not contain a protein stabilizer (e.g., BSA, gelatin), which inhibits coupling of the antibody to the beads. Remove protein stabilizers with the Thermo Scientific Pierce Antibody Clean-up Kit (Product No. 44600, on our website see Tech Tip #55: Remove BSA and gelatin from antibody solutions using Melon Gel).
- Primary amine-containing buffers (e.g., Tris and glycine) inhibit coupling of protein to the magnetic beads. Remove primary amine-containing buffer using dialysis (e.g., Thermo Scientific Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 3mL, Product No. 87730) or desalting (e.g., Thermo Scientific Zeba Spin Desalting Columns, 7K MWCO, 0.5mL, Product No. 89882).
- For optimal results, use an affinity-purified antibody. Although serum may be used, the antibody specific for the antigen of interest may comprise only 1-2% of the total IgG in the serum sample, resulting in low antigen recovery.
- The IP Lysis/Wash Buffer has been tested on representative cell types including, but not limited to, the following cell lines: HeLa, Jurkat, A431, A459, MOPC, NIH 3T3, and U2OS. Typically, 10^6 HeLa cells yield ~10mg of cell pellet and ~3µg/µL or 300µg when lysed with 100µL of IP Lysis/Wash Buffer.
- To minimize protein degradation, include protease inhibitors (e.g., Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail EDTA-free, Product No. 78425) in the preparation of cell lysates.
- The IP Lysis/Wash Buffer is compatible with Thermo Scientific Pierce BCA Protein Assay (Product No. 23225).
- Optimal time for low-pH elution is 5 minutes; if the desired amount of antigen is not obtained, then a second 5 minute low-pH elution may be performed to remove additional bound antigen.

Procedure for Mammalian Cell Lysis

A. Additional Materials Required

- Phosphate-buffered saline (PBS, 100mM sodium phosphate, 100mM NaCl; pH 7.2; Product No. 28372)
- Cultured cells

Protocol I: Lysis of Cell Monolayer (Adherent) Cultures

1. Carefully remove culture medium from confluent cells.
2. Wash the cells once with PBS.
3. Add ice-cold IP Lysis/Wash Buffer to the cells (see Table 2) and incubate on ice for 5 minutes with periodic mixing.

Table 2. Suggested volume of IP Lysis/Wash Buffer to use for different standard culture plates.

Plate Size/Surface Area	Volume of IP Lysis/Wash Buffer
100 × 100mm	500-1000µL
100 × 60mm	250-500µL
6-well plate	200-400µL per well
24-well plate	100-200µL per well

4. Transfer the lysate to a microcentrifuge tube and centrifuge at ~13,000 × *g* for 10 minutes to pellet the cell debris.
5. Transfer supernatant to a new tube for protein concentration determination and further analysis.

Protocol II: Lysis of Cell Suspension Cultures

1. Centrifuge the cell suspension at 1000 × *g* for 5 minutes to pellet the cells. Discard the supernatant.
2. Wash cells once by suspending the cell pellet in PBS. Centrifuge at 1000 × *g* for 5 minutes to pellet cells.
3. Add ice-cold IP Lysis/Wash Buffer to the cell pellet. Use 500µL of IP Lysis/Wash Buffer per 50mg of wet cell pellet (i.e., 10:1 v/w). If using a large amount of cells, first add 10% of the final volume of IP Lysis/Wash Buffer to the pellet and pipette the mixture up and down to mix. Add the remaining volume IP Lysis/Wash Buffer to the cell suspension.
4. Incubate lysate on ice for 5 minutes with periodic mixing. Remove cell debris by centrifugation at ~13,000 × *g* for 10 minutes.
5. Transfer supernatant to a new tube for protein concentration determination and further analysis.

Manual Procedure for the Pierce Direct Magnetic IP/Co-IP Kit

A. Additional Materials Required

- Magnetic stand (e.g., Thermo Scientific Magnabind Magnet for 6 × 1.5mL microcentrifuge tubes, Product No. 21359)
- 1.5mL microcentrifuge tubes
- Ice-cold 1mM hydrochloric acid
- Coupling antibody
- Antigen sample for IP
- Ultrapure water

B. Coupling Antibodies to the NHS-Activated Magnetic Beads

Note: The following protocol is optimized for coupling 5µg of antibody to 25µL of beads, but can be used for coupling 2-10µg of antibody. This procedure can be scaled up to prepare larger quantities of antibody-coupled beads.

1. Vortex the bottle of NHS-activated magnetic beads to obtain a homogeneous suspension. Add 25µL of beads into a microcentrifuge tube. Place the tube on a magnetic stand and collect the beads for 1 minute. Remove and discard the storage solution.
2. Add 400µL of ice-cold 1mM HCl to the tube and gently vortex to mix for 5 seconds. Collect the beads on a magnetic stand. Remove and discard the supernatant.
3. Prepare antibody solution by diluting antibody stock into 10µL of Borate Buffer (0.67M) and adding ultrapure water to bring the final volume to 100µL. The final concentration of antibody will be 5µg/100µL and the final concentration of Borate Buffer will be 0.067M.

   Note: Adjust the antibody solution for antibody amounts other than 5µg per IP.
4. Add 100µL of prepared antibody solution to the beads. Gently mix and incubate on a rotating platform for 30-60 minutes at room temperature; vortex the beads every 10-15 minutes during incubation to ensure that the beads remain in suspension.
5. Collect the beads with a magnetic stand. Remove the flow-through and save for analysis.
6. Add 100µL of Elution Buffer and gently vortex or invert to mix. Collect the beads on a magnetic stand. Remove and discard the supernatant. Repeat this wash once.

   Note: The Elution Buffer removes non-covalently bound antibody.
7. Add 500µL of Quenching Buffer to the beads. Gently mix and incubate on a rotating platform for 30-60 minutes.
8. Collect the beads with a magnetic stand. Remove and discard the supernatant.
9. Prepare 0.5mL of Modified Borate Buffer for each IP reaction by diluting 25µL of IP Lysis/Wash Buffer with 475µL of Borate Buffer (prepared from a Thermo Scientific BupH Borate Buffer Pack, contents dissolved in a final volume of 500mL ultrapure water).
10. Add 500µL of Modified Borate Buffer prepared in Step 9 to the tube and gently vortex or invert to mix. Collect the beads on a magnetic stand. Remove and discard the supernatant.
11. Add 500µL of IP Lysis/Wash Buffer and gently vortex or invert to mix. Collect the beads on a magnetic stand. Remove and discard the supernatant.

C. Antigen Immunoprecipitation

1. Dilute the lysate solution (see the Procedure for Mammalian Cell Lysis Section) with IP Lysis/Wash Buffer to 1-2mg/mL.
2. Add 500µL of diluted lysate solution to the tube containing the antibody-coupled magnetic beads and incubate for 2 hours at room temperature on a rotator or mixer. Gently vortex the beads every 15-30 minutes during incubation to ensure the beads stay in suspension.
3. Collect the beads on a magnetic stand. Remove the unbound sample and save for analysis.
4. Add 500µL of IP Lysis/Wash Buffer to the tube and gently vortex or invert to mix. Collect the beads on a magnetic stand. Remove and discard the supernatant. Repeat this wash once.
5. Add 500µL of ultrapure water to the tube and gently vortex or invert to mix. Collect the beads on a magnetic stand. Remove and discard the supernatant.
6. Add 100µL of Elution Buffer to the tube. Incubate for 5 minutes at room temperature on a rotator or mixer. Magnetically separate the beads and save the supernatant containing the target antigen. To neutralize the low pH, add 10µL of Neutralization Buffer for each 100µL of eluate.

   Note: If the desired amount of antigen is not obtained, then a second 5 minute low-pH elution can be performed to recover additional bound antigen.

Automated Procedure for the Pierce Direct Magnetic IP/Co-IP Kit

A. Additional Materials Required

- KingFisher® Flex System with 96 deep well head (Product No. 5400630) or KingFisher 96 System (Product No. 5400500)
- Thermo Scientific Microtiter Deep Well 96 Plates, V-bottom, polypropylene (100-1000µL; Product No. 95040450)
- KingFisher Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)
- 1.5mL microcentrifuge tubes
- Ice-cold 1mM HCl
- Coupling antibody
- Antigen sample for IP
- Ultrapure water

B. Automated Protocol for Antibody Coupling and Antigen Immunoprecipitation

Note: The following protocol is designed for use with the KingFisher Flex or KingFisher 96 Instrument. The protocol can be modified according to your needs using the Thermo Scientific BindIt Software provided with the instrument.

Note: Lysate solution in the protocol is from the Procedure for Mammalian Cell Lysis Section.

1. Enter the "Direct Immunoprecipitation" protocol from Table 3 into the BindIt® Software on an external computer.
2. Transfer the protocol to the KingFisher Flex or KingFisher 96 Instrument from an external computer. See the BindIt Software user manual for detailed instructions on importing protocols.
3. Set up plates according to Table 3.

Table 3. Pipetting instructions for the automated protocol using Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume	Time/Speed
1	Beads	NHS-Activated Beads	25µL	Collect Beads
		Ice-cold 1mM HCl	175µL	
2	HCl Wash	Ice-cold 1mM HCl	400µL	5 seconds/Slow
3	Coupling	Antibody Sample	100µL	30 minutes/Slow
4	Elution Buffer Wash 1	Elution Buffer	100µL	10 seconds/Slow
5	Elution Buffer Wash 2	Elution Buffer	100µL	10 seconds/Slow
6	Quench	Quenching Buffer	500µL	30 minutes/Slow
7	Wash 1	Modified Borate Buffer	500µL	30 seconds/Slow
8	Wash 2	IP Lysis/Wash Buffer	500µL	30 seconds/Slow
9	Bind	Lysate solution	500µL	2 hours/Slow
10	IP Wash 1	IP Lysis/Wash Buffer	500µL	30 seconds/Slow
11	IP Wash 2	IP Lysis/Wash Buffer	500µL	30 seconds/Slow
12	IP Wash 3	Purified Water	500µL	30 seconds/Slow
13	Elution	Elution Buffer	100µL	5 minutes/Slow
14	Tip Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	-	-

4. Select the protocol using the arrow keys on the instrument keypad and press Start. See the KingFisher Flex or KingFisher 96 Instrument user manual for detailed information.
5. Slide open the door of the instrument's protective cover.
6. Load the Tip Plate (plate #14) and press Start. The instrument places the Tip Comb onto the magnet head.
7. Remove the Tip Plate.
8. Load plates #1-8 into the instrument according to the protocol requests; place each plate in the same orientation. Confirm each action by pressing Start.
9. After sample processing through plate #8, the instrument will pause and instruct to remove each individual processed plate while simultaneously loading each of the remaining six plates, plates #9-14. For example, remove plate #1 and load plate #9 into that position. Confirm each action by pressing Start.
10. After sample processing, remove the plates as instructed by the instrument display. Press Start after each plate. Press Stop after removing all of the plates.

Notes:

- Load ice-cold 1mM HCl and beads in plates #1 and 2 immediately before loading onto the instrument to ensure minimal hydrolysis of the beads.
- Neutralize the low-pH elutions by adding 10µL of Neutralization Buffer for each 100µL of eluate directly to each well immediately after incubation.
- If using fewer than 96 wells, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.

Troubleshooting

Problem	Possible Cause	Solution
Low coupling efficiency	Primary amine-containing buffer was not completely removed before coupling	Dialyze or desalt sample to completely remove Tris and glycine
	Protein addition was delayed	Immediately mix protein with beads after washing
	Wrong pH for the coupling buffer	Use non-amine containing buffer at pH 7-9 for the coupling buffer
Insoluble protein in coupling buffer	Protein was hydrophobic	Dissolve protein in coupling buffer containing up to 20% DMSO
Magnetic beads aggregate	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	Beads were not thoroughly mixed before and/or during incubation	
Beads aggregate during the coupling process	Proteins on bead surface adhered to tube plastic	After quenching, add 0.05% detergent (e.g., Tween®-20 Detergent) to the washes **Note:** Do not use detergent in the coupling step

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Antibody detected with the eluted antigen	Non-bound antibody was not sufficiently removed with the washes following the coupling step (Step 5)	Increase the number of Elution Buffer washes after coupling
	The antibody-coupled beads were treated with a reducing agent (e.g., DTT) during the IP or elution steps, which reduced the antibody and eluted antibody fragments or subunits that were not covalently linked to the beads	Use buffers that do not contain reducing agents
Protein does not elute	Elution conditions were too mild	Increase incubation time with elution buffer to 10 minutes or use a more stringent elution buffer
	Component in IP Lysis/Wash Buffer interfered with antibody-antigen binding	Perform IP and washes using an alternative buffer (e.g., 0.5% CHAPS in TBS)
Low amount of recovered protein	Protein degraded	Add protease inhibitors
	Not enough magnetic beads were used for capture	Increase the amount of magnetic beads used for capture
	Sample had an insufficient amount of target protein	Increase amount of antigen sample
Recovered protein was inactive	Elution conditions were too stringent	Use a milder elution buffer (e.g., Thermo Scientific Gentle Elution Buffer, Product No. 21034)

Additional Information

Visit *www.thermoscientific.com/pierce* for additional information relating to this product including the following:

- Frequently Asked Questions
- Tech Tip # 43: Protein stability and storage
- Tech Tip #55: Remove BSA and gelatin from antibody solutions using Melon Gel
- Tech Tip #75: Measure protein bound to Pierce NHS-Activated Magnetic Beads
- Visit *www.thermoscientific.com/kingfisher* for information on KingFisher Products.
- In the U.S.A., purchase KingFisher Supplies from VWR. Outside the U.S.A., contact your local Thermo Fisher Scientific office to purchase KingFisher Supplies.

Related Thermo Scientific Products

88826	**Pierce NHS-Activated Magnetic Beads,** 1mL
88827	**Pierce NHS-Activated Magnetic Beads,** 5mL
88802	**Pierce Protein A/G Magnetic Beads,** 1mL
88803	**Pierce Protein A/G Magnetic Beads,** 5mL
88804	**Pierce Protein A/G Magnetic IP/Co-IP Kit**
88805	**Pierce Crosslink Magnetic IP/Co-IP Kit**
88816	**Pierce Streptavidin Magnetic Beads,** 1mL
88817	**Pierce Streptavidin Magnetic Beads,** 5mL
88821	**Pierce Glutathione Magnetic Beads,** 4mL

88822	**Pierce Glutathione Magnetic Beads,** 20mL
23235	**Micro BCA Protein Assay Kit**
44600	**Pierce Antibody Clean-up Kit**

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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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